# Comparative phytochemical studies of selected medicinal plants in Gwalior (M.P.) region

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## Abstract

The present study was aimed to evaluate the phytochemical constituents of the leaf extracts of *Barleria prionitis* L., *Cassia tora* (L) Roxb., *Catharanthus roseus* (L.) G. Don. and *Lantana camara* L.Six medicinal plants of ethanobotanical importance belonging to families Acanthaceae, Caesalpiniaceae, Apocynaceae, Verbenaceae, Myrtaceae and Cucurbitaceae were investigated for the presence of alkaloids, flavanoids, flavonols, flavones and flavanols, saponins and steroids in their aqueous extracts. *Barleria prionitis* L., *Cassia tora* (L) Roxb., *Catharanthus roseus* (L.) G. Don., *Lantana camara* L. showed positive tests for alkaloids. All plants gave negative results for cardenolides. *C. tora*, *C. roseus*, *L. camara* showed positive results for alkaloids and flavonoides. *B. prionitis*, *C. tora* gave positive tests for flavonols and flavonols and flavonones. *C. roseus*, *L. camara* showed positive tests for alkaloids, flavonoids, flavanols and flavonones.

**Key words**: Phytochemical, *B. prionitis*, *C. tora*, *C. roseus*, *L. camara*, alkaloids, aqueous

## Introduction

The World Health Organization has estimated that 80% of the inhabitants of the world rely mainly on traditional plants for their primary health care needs and it may be presumed that a major part of traditional healing involves the use of plant extracts or their active principles. Medicinal plants have been traditionally used for different kinds of infection diseases [Chitravudu *et.al*., 2009] However, several studies have indicated that medicinal plants contain compounds, e.g. peptides, unsaturated fatty acid, aldehydes, flavonoids, alkaloids, essential oils, phenols and water or ethanol soluble compounds. These compounds are significant therapeutic application against pathogens, including bacteria, fungus and viruses [Singh et. al. 2010]. These secondary metabolites produced by plants are organic chemicals of

high structural density which play different functions including chemotherapeutic, bactericidal, bacteriostatic and antimicrobial functions [Purohit and Mathur, 1999].

*Lantana camara* belongs to the family Verbenaceae commonly known as Ghaneri. The leaves of this plant were used as an antitumeral, antibacterial, and antihypertensive agent [Taubi *et. al*. 2007]. In herbal medicine, infusions of the leaves and other plant parts are used as an anti-inflammatory [Odeyapo *et. al*., 1997]. *Barleria prionitis* belongs to family Acanthaceae. Its leaves are used to promote healing of wounds and to relieve joint pains and toothache [Parrotta, 2001]. Because of its antiseptic properties, extracts of the plant are incorporated into herbal cosmetics and hair products to promote skin and scalp health [Prakruti, 2002]. *Catharanthus roseus* belong to family Apocynaceae known as sadabahar. It is cultivated mainly for its alkaloids, which are having anticancer activities [Jaleel *et al*., 2008]. Several research groups have shown that *Catharanthus roseus* has a high potential for many varieties of medicinal properties, such as antibacterial [Carew and Patterson 1970], antifungal [Jaleel *et al*. 2007] and antiviral [Farnsworth *et al*. 1968]. *Cassia tora* belong to family Apocynaceae commonly known as chakoda. The extracts of *C. tora* have been used as a remedy for various skin ailments, rheumatic disease and as laxatives [Hooker, 1879; Kirtikar and Basu, 1975; Jain, 1968]. The extract of *C. tora* leaves has been found to possess significant hepatoprotective activity and anti-inflammatory activity [Maitya *et.al*., 1997, 1998].

**Methods:**

**Phytochemical studies (Qualitative chemical analysis):**

The plant leaves were air dried in laboratory and ground into uniform powder and stored in container. These plant parts were subjected to qualitative chemical screening for the identification of the various classes of active chemical constituents using standard prescribed methods [Amarsingham *et al.* 1964; Gibbs, 1974].

**(i) Test for Alkaloids**:

5 gm of dried powder was kept in 50ml of 10% ethanol for 48 hours at room temperature with occasional shaking. The extract was filtered and distilled water vaccum. The dried concentrated extract was acidified with 25ml of 0.1 N$H_2SO_4$. The acid extract was centrifuged and the clear supernatant was tested with Mayer's, Wagner's and Dragendorff's reagent.

**(a) Mayer's Reagent:**

1.358 gm of Mercuric Chloride and 5gm of Potassium Iodide dissolved 100ml of water. Both the solutions were mixed and diluted to 100ml with distilled water. To a little of the test filtrate, taken in a watch glass, a few drops of the above reagent were added. Formation of creamy coloured pigment showed presence of alkaloids.

**(b) Wagner's reagent:**

2 gm of iodine and 6 gm of Potassium Iodide dissolve in 100ml of distilled water. When few drops of this reagent were added to the test filtrate, a brown precipitate was formed indicating the presence of alkaloids.

**(c) Dragendorff's reagent**:

0.850 gm basic Bismuth nitrate is dissolved in a mixture of 10ml acetic acid and 40 ml distilled water. 8 gm of Potassium Iodide dissolved in 20 ml of distilled water. These stock solutions were mixed together. The above reagent was sprayed on Whatmann filter paper no.1 and the paper was dried. The test filtrates after basification with dilute Ammonia was extracted with chloroform. The chloroform extract was applied on the filter paper, impregnated with Dragendorff's reagent, with the help of an orange red colour on the paper indicated the presence of alkaloids.

**(ii) Test for Anthraquinones**:

They occur as free anthrons derivatives or as glycosides.

a) The powder was boiled with sulphuric acid for one hour, cooled and filtered. To the filtrate was added Chloroform. The mixture was vigorously shaken and allowed to stand, organic layer gets separated. Ammonia was added to organic layer slowly. Development of red, pink, or violet colour indicates the presence of Anthraquinones. This is to detect glycosides.
b) The powder was extracted with 80% ethanol. The extract was dried. Residue was dried. Residue was dissolved in distilled water, filtered and shaken with benzene in separating funnel 5ml of ammonia added to the benzene layer. Red ammonia layer indicates the presence of Anthraquinones. This is to detect Anthraquinones.

**(iii) Test for Cardiac glycosides (Cardenolides):**

Fresh tissue was extracted with rectified spirit. To the extract 10% solution of Sodium Hydroxide and 0.3% solution of Nitropruside were added. Appearance of transient pinkish red colouration indicates the presence of Cardenolides.

**(iv) Test for Flavonoids**:

Different tests were carried out for different types of flavonoids. Tests were carried with dry sample.

**(a) Flavonoids (Shinoda test):**

To the extract, a piece of Magnesium ribbon and Hydrochloric acid were added. Purple, red, pink or orange colour developed, which confirm flavonoids.

**(b) Flavanonols:**

If deep colour developed with Shinoda test, then instead of Magnesium ribbon, Zinc powder was added with Hydrochloric acid. Deep magenta colour developed which confirmed flavononols.

**(c) Flavonols:**

To the extract a pinch of Boric acid and few drops of Acetic acid were added. Bright yellow colour with green fluorescence indicated flavonols.

**(d) Flavones and Flavanols:**

Firstly extract was dissolved in Sulphuric acid to give yellow solution and the flavanones produced lively orange to crimson colours. To the extract few drops of Sulphuric acid were added and colour was noted. This further confirmed the presence of Flavones, Flavanols and Flavanones.

**(e) Rao and Sheshadri test:**

To the extract, few drops of Concentrated Nitric acid were added. Brilliant blue colour developed confirmed the presence of phlorglucinol derived flavanones.

**(f) Test for Leucoanthocyanin**:

In this test, fresh as well as dry sample can be used. 0.5 gm of sample was heated with 2N HCL and given water bath for about 20 minutes. The extract was allowed to cool down at room temperature, filtered and to the filtrate 5ml of Iso-amyl alcohol was added. On the presence of Leucoanthocyanins, the Iso-amyl layer became red. This was noted as '++ve'. When the Iso-amyl layer became pink, the reaction was noted as weak '+ve'; no colour to Iso-amyl layer was noted as '−ve'. In some cases, the Iso-amyl layer became reddish brown. This was denoted as 'thought to be a doubtful case'. The darkening of the colour of solution during boiling (particularly becoming brown) was also noted. The darkening of solution was usually associated with acubin type glycosides in the plant material.

**(g) Irridoids / Acubins:**

Fresh as well as dry leaves were used for these tests. Leaves were powdered and 5 ml of 1% aqueous hydrochloric acid was added. Extraction was carried out for 6

hours. To the 0.1 ml of extract, 1ml of Trim hill reagent was added. The tube was heated for short time in a flame and colour change was noted. Production of blue, red, violet colour indicates the presence of acubins / irridoids.

**Trim Hill reagents:** 10 ml of acetic acid + 1 ml 2% aqueous copper sulphate + 0.5 ml concentrated hydrochloric acid**.**

**(vi) Test for Simple Phenolics:**

Plant powder was extracted with aqueous ethanol overnight. To the extract 1-2 drops of 1% aqueous Ferric chloride was added. Development of specific colours was indicative of the presence of Phenol.

**(vii) Test for Saponins:**

The powder was extracted with boiling water. After cooling, the extract was shaken vigorously to froath and was then allowed to stand for 10-15 minutes. The persistent froath of 2cm high considered as presence of Saponins.

**(viii) Test for Steroids:**

2ml of acetic anhydride was added to 0.5 gm ethanolic extract of each sample with 2ml $H_2SO_4$. The colour changes from violet to blue or green in some samples indicating the presence of steroids.

**Table 1**: Comparative phytochemical study of *B. prionitis*, *C. tora, C. roseus*, *L.camara*, with aqueous extracts

| Components | *B. prionitis* | *C. tora* | *C. roseus* | *L. camara* |
|---|---|---|---|---|
| Alkaloids | - | + | + | + |

| | | | | |
|---|---|---|---|---|
| Anthraquinone | - | + | _ | _ |
| Cardenolides | - | _ | _ | _ |
| Flavonoids | - | + | + | + |
| Flavononols | _ | + | _ | + |
| Flavonols | + | + | _ | + |
| Flavones & Flavanols | + | + | + | + |
| Flavonones | - | _ | _ | _ |
| Iridioides | + | _ | _ | + |
| Leucoanthocyanin | _ | + | _ | + |
| Phenolics | - | + | _ | _ |
| Saponins | + | _ | + | + |
| Steroids | - | + | _ | + |

**Results and Discussion:**

Aqueous extract of *B. prionitis* showed no inhibition potency. Reports indicate that the antifungal activity is due to presence of different compounds in the extract, including flavonols, flavones, flavanols, irridoids and saponins [Chen *et al*., 1998; Burkill, 1985].This species showed positive tests for alkaloids, flavonols, flavones, flavanols, saponins.

Anthraquinones present in *C. tora* leaves exhibit antimicrobial activity [Rios *et al*., 1987; Diaz *et al*., 1988; Mukherjee *et al*., 1996; Maity, 1999; Goyal *et al*., 2007; Rai and Abdulahi, 1978]. Flavonoids are known to be synthesized by plants in response to microbial infections [Dixon *et al*., 1983]. Acharya and Chaterjee [1975] isolated chrysophanic acid- 9- anthrone, the major antifungal principle in *C. tora*. Leucoanthocyanin have been reported in leaves of *C. tora* [Sofowora, 1982]. They are one of the most powerful bioflavonoids. It improves the strength of the blood vessels including varicose veins. Alkaloids, anthraquinone, flavonoids, flavononols, flavanols, flavonols, flavones, leucoanthocyanin, saponin and steroids were found to be present in its leaf powder.

About 150 alkaloids have now been isolated from *C. roseus* some important are ajmalicine, lochnerine, serpentine and tetrahydroalstonine; occur in various genera of this family. Presences of alkaloids,

flavonoids, flavones, flavanols, favonols, saponins and phenolics have been found in various concentrations in the plants of this family [Mustafa and Verpoorte 2007]. The species exhibited positive test for alkaloids, flavonols, flavanols, flavones, irridoides, saponins, phenolics and steroids

The medicinal properties of this species have attributed due to presence of an antimicrobial flavonoid 'umuhengerin' isolated from the leaves of *L. camara* have also been reported to contain flavononols, flavonols, flavonoids and flavones [Anonymous, 2005; Barre *et al*., 1997; Majekodunmi *et al*. 2002 and Ghisalberti, 2000]. Irridoid glycosides have also been reported from this species. They appear to be the characteristic features of the family. Various compounds such as alkaloids, flavonols, flavones, flavanols, flavonoids, leucoanthocyanin, steroids, saponin, irridoides were present in it.

1) Anthraquinones, flavonoids, saponins and steroids were noticed to be present *L. camara*.
2) Alkaloids, anthraquinone were observed in *B. prionitis* leaves.
3) Irridoides, leucoanthocyanin were present in *B. prionitis*, *C. tora*, *L. camara*.

**Conclusions :**

The research work showed the presence of various phytochemical constituents in *C. tora*, *L. camara* in aqueous extract. The selected plants are the source of secondary metabolites. *C. tora*, *L. camara* plant shows the variation in its constituents which are useful in the manufacturing of drugs. These types of studies have vital role because of the commercial and research interest. Further research is necessary to isolate and determine the identity of the active compound.

**Acknowledgement:**

The authors are thankful to Professor Dr. A. K Jain [Department of Botany, Jiwaji University, Gwalior] for providing the laboratory facilities and other necessary support.

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# Isolation of potent zinc and phosphate solubilizing bacterial isolates from garden soil

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## Abstract

Human population is continuously increasing; as a result there is a rapid demand of food throughout the world. More yield and increase in crop production as well as increase in soil fertility without effecting the environment are major concern of today's world. Agriculture contributes most to the increasing amount of chemical pollutants via excessive use of synthetic chemical fertilizers and pesticides, which causes environmental damage with potential risks to human health. The whole world is shifting to an organic based sustainable agriculture. It is necessary to preserve the nature's wealth for the future generation. The population of world is increasing rapidly and is creating pressure on the existing land area for fiber, fuel, food and raw materials. The above said problem may be resolved by application of plant growth promoting rhizobacteria capable of solubilizing zinc and phosphate in the soil. Thus, the aim of this study was isolate potent bacterial strains from the soil sample having capacity of zinc and phosphate solubilization.

**Keywords: PGPR; Soil; Zinc; Phosphate; Biofertilizer**

## 1. Introduction

Agriculture contributes in the major part of the economy of the country in many developing countries and plays a vital role. It ensures the food security and employment [1]. There is a large competition for producing more crop yield through adopting more and more advanced, improved and intensive agronomic practices. Use of fertilizers in large amount for crop yield is adversely affecting the health of soil and soil is continuously degrading. Sustainable agriculture practice is important to meet the demand of today's world without polluting

the environment. By mean of conventional method of agriculture, we can't meet the demand of future. Plant vigor and fertility of soil can be increased by solubilizing various micronutrients with help of bacterial strains. In the last few decades, the agriculture policy in India has undergone a major change through diversification and emphasis on sustainable production system [2]. Zinc is required for growth as well as metabolism of various micro-organisms and plants. It is required in small but critical concentration to allow several key plant physiological pathways to function normally. These pathways have important roles in photosynthesis, sugar formation, protein synthesis, fertility and seed production, growth regulation and defense against disease [3]. Deficiency of zinc affects the morphological function of plants and adversely affects health and production. It also leads to lower yielding of crops or even crop failure and frequently in poor quality crop production. Zinc has its role in nutrition and physiology of both prokaryotic and eukaryotic organisms. Availability of the zinc in soil and aquatic environment can affect the productivity and diversity of ecosystem. Zinc is also present in enzyme system and also found as a co-factor and metal activator of many enzymes. Zinc deficiency in fungi and bacteria is accompanied by impairment of the formation of pigments such as melanin, chrisogenin, prodigiosin, subtilin and others. Many bacterial enzymes contain zinc in its structure and the active sites [4]. Zinc is found in several forms in soil such as sulphate, olivine, augite, biotite etc. This form of zinc has its role in conversion of such unavailable sources into available one. Microorganism such as *Rhizobacteria* plays an important role in such conversion. Zinc solubilizing bacteria are those which are capable of solubilizing the insoluble zinc compound or minerals in agar plate as well as in soil [5]. Phosphorus is an essential element for plant growth. It is available in limited quantity in soluble form. Phosphorus often limits the growth and development of plants. Generally, very high amount of phosphorus is present in soil. However, most of the phosphorus is insoluble and not available to plants for its growth. Several *Rhizospheric* bacteria are capable of transforming soil phosphorus to the form easily available to plants. Several bacterial genuses like *Pseudomonas, Bacillus, Rhizobium*, and *Enterobacter* are phosphate solubiliser. *Pseudomonas fluorescens* is relatively new bacterial strain which solubilizes phosphorus. Organic matter

derived from dead and decaying plant debris is rich in organic sources of phosphorus. However, plants are able to utilize phosphorus from soil only in the free available from. Soil phosphates are rendered available either by plant roots or by soil microorganism. Therefore, phosphate – dissolving soil organisms play a part in correcting phosphorus deficiency of crop plants [6]. Excessive use of chemical fertilizers have adverse effects on soil microorganism, it affects the fertility status of soil and also pollutes environment [7]. The application of these fertilizers often leads to reduction in pH and thus making the zinc and phosphate unavailable to crops and thus leads to reduction in crop yield. Besides being costly, the production of chemical fertilizers depletes non-renewable resources, the oil and natural gas used to produce these fertilizers, and poses human and environmental hazards [2]. Plant growth promoting Rhizobacteria (PGPR) plays an important role in the sustainable agriculture. The increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticides use is a big challenge nowadays. The use of PGPR has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth through either a direct or indirect mechanism. The mechanisms of PGPR include regulating hormonal and nutritional balance, inducing resistance against plant pathogens, and solubilizing nutrients for easy uptake by plants. In addition, PGPR show synergistic and antagonistic interactions with microorganisms within the rhizosphere and beyond in bulk soil, thus indirectly boosts plant growth rate. There are many bacteria species that act as PGPR.

## 2. Materials and Methods

### 2.1. Sample collection

Soil sample was collected from Rhizospheric soil of plants planted in kitchen garden following the procedure prescribed by Rai et al. 2014. Soil samples were serial diluted and dilution of $10^{-5} – 10^{-7}$ was plated on Nutrient agar medium.

### 2.2. Isolation and purification of isolates

Isolation and purification of bacterial strain was performed by sub culturing the obtained colonies on Nutrient agar media (NAM) having composition (g/L) Peptone 05.0 gm; Beef extract 03.0 gm; Sodium chloride 05.0 gm; Agar15.0 gm; Distilled water 1000 ml and the pH was maintained to 7.

### 2.3. Morphological characterization

Morphological characterization of the isolates was done using the standard

techniques of Microscopic observation based on colony morphology (Size, shape, colour, elevation, texture, opacity and margin).

**2.4. Microscopic observation**

For cell morphology and differentiating in bacterial strain, isolated bacterial strains were studied microscopically by Gram staining procedure. The prepared slide was observed under oil immersion (100x) for bacterial morphology, shape and mode of arrangements.

**2.5. Zinc solubilization test:**

The zinc solubilizing activity of each *isolate was tested* in liquid broth as well as on solid agar plate. Medium used was Pikoviskaya's medium (Yeast Extract 0.050 g; Dextrose 10 g; Calcium Phosphate 0.5 g; Ammonium Sulphate 0.50 g ;Potassium Chloride 0.20 g; Magnessium Sulphate 0.10 g; Magnese Sulphate 0.0001 g; Ferrous Sulphate 0.0001 g in g/L). Quantative study of zinc solubilization was studied in 500 ml of conical flask containing 250 ml Pikoviskaya's broth medium. In broth assay method first we have to prepare a Pikoviskaya's broth media. The Broth was inoculated with loopful culture of overnight grow bacterial inoculum. Then incubated for 5 days at 160 rpm in a shaker incubator at 28$^{o}$C. After incubation, broth culture were centrifuged at 8000 rpm for 10 min and filtered through Whatman No.42 filter paper. In plate assay method, first Pikoviskaya's Agar media was prepared. Then sterilization and plating were done. Freshly grown bacterial cultures were spot inoculated into the agar plate. The spotted plates were incubated at 28$^{o}$C for 48 hrs in incubator. The clearing zone or haloes around colonies were formed; the diameter of the haloes around the colony and colony diameter were measured. Subsequently the plates were flooded with methyl red solution to observe acid production by bacteria. The change of clear zone to red is an indication of acid production.

**2.6. Phosphate Solubilization:**

To detect the phosphate solubilisation potential of isolated bacterial strain, first a Pikovaskaya's agar media is prepared. Then sterilization and poring were done. Bacterial cultures were spot inoculated on the Pikovaskaya's agar medium and incubate at 28$^{o}$C for 2-3 days. Clear zone formed around bacterial colony after the incubation. Clear zone formation indicated the positive results. Diameter of the halo zones around the colony and the colony diameter were measured.

## 3. Results

### 3.1 Isolation of bacteria

Bacterial isolates were obtained from the rhizospheric soil by $10^{-5}$, $10^{-6}$ and $10^{-7}$ serial dilution. Among the bacterial isolates, five Isolates showed characteristics of ***Pseudomonas*** species on Nutrient agar media.

### 3.2. Microscopic identification

All the isolates were identified macroscopically by studying colony morphology based on their appearance on individual Nutrient agar medium plate.

Table 1- Colony charaterization of the isolates on zinc supplemented Nutrient agar medium.

| Isolate No | No. of Col. | Size (mm) | Shape Of colony | Colour | Eleva-tion | Texture | Margin | Opacity |
|---|---|---|---|---|---|---|---|---|
| **1** | 71 | 10 | lrregular | Greenish | Flat | Smooth | Undulate | Translucent |
| **2** | 63 | 7 | lrregular | Greenish | Flat | Smooth | Undulate | Translucent |
| **3** | 52 | 6 | lrregular | Greenish | Flat | Smooth | Undulate | Translucent |

Table 2- Colony characheteristics of isolates on phosphate supplemented Nutrient agar medium.

| Isolate No. | No.of Col. | Size (mm) | Shape of colony | Colour | Eleva tion | Texture | Margin | Opacity |
|---|---|---|---|---|---|---|---|---|
| **1** | 7 | 5 | lrregular | Orengus | Flat | Smooth | Undulate | Translucent |
| **2** | 12 | 3 | lrregular | Orengus | Flat | Smooth | Undulate | Translucent |
| **3** | 5 | 7 | lrregular | Orengus | Flat | Smooth | Undulate | Translucent |

**A**

**B**

Fig. 1 – Colony characteristics of isolates on zinc and phosphate supplemented nutrient agar medium.

### 3.3. Microscopic identification of bacterial isolation

Microscopic examination was done on the basis of gram staining. Isolates were found Gram negative and short rods.

### 3.4. Phosphate and Zinc solublization potential

Two metods is used for the determination of zinc solubilization potential. These two methods are Broth assay methods and Agar assay methods. For the Quantative study of zinc and phosphate Pikoviskaya,s Broth and Pikoviskaya,s Agar is used as solid medium respectively in the experiment. The zinc solubilizing activity of each *Psedumonas fluorescence* isolate in liquid broth was determined quantitatively. The concentration of zinc in supernatant was estimated in atomic absorption spectrophotometer at 600 nm for OD. The OD of the supernatant is measured and the weight of the biomass was observed to detrmined the zinc solubilizing potential.

The clearing zone or haloes around colonies were formed on a solid medium of bacterial plate. The diameter of the haloes around the colony and colony diameter were measured. Subsequently the plates were flooded with methyl red solution to observe the acid production by bacteria. The change of clear zone to red is an indication of acid production. The zinc solubilization ability is measured by calculating the diameter of colonies and the halo zones.

Table 3. Zinc solubilization potential of isolates

| **Isolate** | **Diameter of Halozone** | **Diameter of Culture** |
|---|---|---|
| **1** | **1.3 cm** | **0.6 cm** |
| **2** | **1.3 cm** | **0.6 cm** |
| **3** | **1.4 cm** | **0.7 cm** |
| **4** | **2.2 cm** | |

| | | |
|---|---|---|
| | | **1.9 cm** |

Table 4. Phosphate solubilization potential of isolates

| | |
|---|---|
| **1.5 cm** | **0.8 cm** |
| **1.4 cm** | **0.6 cm** |
| **1.2 cm** | **0.7 cm** |
| **1.2 cm** | **1.9 cm** |

**4. Discussion**

Plant growth promoting and biocontrol activities of rhizobacteria have been reported by numerous studies in last three decades (3;8). However, the isolates have seldom been applied to elevate the growth and yield of host pants. The application has also been limited due to inconsistency in results of laboratory ,greenhouse and field studies (Mishustin and Naumova, 1962). Production of indole acetic acid (IAA) and soluble phosphate are the mostcommon mechanisms of action implicated in PGPR andindeed microbes demonstrating these attributes arewidespread in rhizosphere. The two isolates found potent in this study were found gram negative, short rods, and fluorescent green in appearance showed the presence of pseudomonas species, were potent for significant amount of IAA, phosphate solubilization and siderophore production. Production of ammonia, phosphate solubilization, siderophore production, and HCN production was most frequently encountered by all the isolates. Another important trait of PGPR, that may indirectly influence the plant growth, is the production of siderophores. They bind to the available form of iron $Fe^{3+}$ in the rhizosphere, thus making it unavailable to the phyto-pathogens and protecting the plant health. In the present, investigation, isolates of *Pseudomonas* spp. showed multiple PGP activities. Several studies have demonstrated that production of siderophores, other secondary metabolites and lytic enzymes by *Pseudomonas* strains was most effective in controlling the plant root pathogens further studies on the performance of these isolates and their mutants on the growth of plant will uncover the mechanism and potential of these PGPR exhibiting multiple plant growth promoting traits.

**5. Conclusion**

The current study aims to exploit phosphate solubilizing bacterial communities in the plant growth promotion. Since there are several factors, inhibiting the growth and development of plants, plant growth promoting bacteria with different attributes facilitating the root and shoot growth. Plant growth promotion by PGPR is a well known phenomenon and this growth enhancement is due to certain rhizobacteria. Here in this report two bacterial strains *P.*

*aeruginosa* and *P. fluorescens* were successfully isolated and have the capability of Phosphate and zinc solubilization. The isolate *P. fluorescens* was found more potent for PGPR activity and further large scale mass production of this isolate may be carried for its commercialization purpose.

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# Promises of nanomaterials as antimicrobial agents: A Review

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**Abstract:**

Prevalence of multi-drug resistant strains of pathogenic bacteria poses serious threat to human health. The over usage of antibiotics has led to the evolution of resistant pathogens are. This has created an urgent need to develop a new generation of antimicrobial agents. Nontraditional antimicrobial agents have been of tremendous interest in overcoming this problem of multidrug resistance developed by several pathogenic microorganisms against most of the commonly used antibiotics. These antimicrobial agents must be effective, safe and can be used for the cure of multidrug-resistant microbial infections. In recent times a lot many properties have been identified in metallic nanoparticles. They can offer effective solutions for these challenges. Several classes of antimicrobial nanoparticles have proven their effectiveness for treating antibiotics resistant infectious diseases. This review summarizes emerging efforts in combating against infectious diseases, particularly using antimicrobial NPs as new tools to tackle the current challenges in treating infectious diseases.

**Keywords:** Drug resistance, Nanoparticles, Pathogenic bacteria, Environment.

## Introduction

The presence of microbial pathogens in environmental reservoirs is a well known fact. These pathogens harbor virulent factors, responsible for the deadly diseases. Antimicrobial therapy has got boom and evolved drastically to cure such infectious diseases. The easy availability and indiscriminate use of antibiotics in clinical infections are the factors that contribute to the emergence and spread of multi-drug resistance in bacteria. In addition, the dissemination of antibiotic resistance genes among human and non-human pathogens is the paradigm for horizontal gene transfer on a global scale. It is likely that close contact of the human population with surface and potable water can enrich the environmental gene pool of pathogens

and lead to emergence of new pathogenic variants. In India and other developing countries, pathogen diagnostics based on antimicrobial agent resistance and virulence gene profiles of *E. coli* pathotypes particularly ETEC and EHEC of surface and potable water resources is not well established [1].

*Salmonellae* are one of the most common causes of water-borne illness in humans. Enteric fever in humans is most commonly caused by *Salmonellae*. *Salmonellae*, usually acquired by the consumption of contaminated water and food have been a major human pathogen since decades. In India, the typhoid occurs with an incidence ranging from 102 to 2,219 per 100,000 populations [2]. With the frequent use of antibiotics to kill *Salmonellae* in previous decades, the pathogen has evolved resistance mechanism to combat against them. As a result, the multidrug-resistant (MDR) *Salmonellae* strains have been prevalent in environment and spread worldwide, resulting in high rates of morbidity and mortality. The extensive use of antibiotics have generated and disseminated drug-resistant *S.* Typhi in the environment and potable water drinking system. The emergence of MDR *S.* Typhi strains to existing antibiotics such as ampicillin, chloramphenicol and co-trimoxazole has complicated the treatment of typhoid fever [3]. This leads to necessity for the development of potential new alternative materials in order to combat this problem.

## 2. Nanomaterials as potential antimicrobial agents

Nanomaterials have successful impact on biology and medicine. Due to the large surface-to-volume ratio the surface activity of nanoparticles (NPs) is higher, providing the ease of surface modification of NPs for enhanced aqueous solubility, biocompatibility or bio-conjugation. In drug targeting research, there is a need for the use of an alternative agent which does not generate resistance and presents a good bactericidal property. A number of studies have been performed to demonstrate the antimicrobial activity of silver nanoparticles (AgNPs). Unfortunately, due to the potent toxicity and cytotoxicity of silver AgNPs, they have not been recommended for the practical use. A potential alternative to this are the gold nanoparticles, which is biocompatible and donot pose toxic effects even when administered into the

cells. Due to their ability to interact with microorganisms GNPs can act as antibacterial agents. The synergistic effects of GNPs and GNP coated drugs can minimize the treatment durations and side effects of drugs with reference drugs and are potential thrust area to be explored.

### 2.1. Silver nanoparticles

Silver has a strong antimicrobial potential, which has been used since the ancient times. But with the advent of antibiotics progress, the medical applications of silver as antimicrobial were declined. Antimicrobial effects of silver can be increased by manipulating their size at nanolevel. Because of their change in physiochemical properties, silver nanoparticles have emerged as antimicrobial agents owing to their high surface-area-to-volume ratio and the unique chemical and physical properties. Silver nanoparticles having size in the range of 10– 100 nm showed strong bactericidal potential against both Gram-positive and Gram-negative bacteria .The bactericidal activity of silver nanoparticles against the pathogenic, MDR as well as multidrug susceptible strains of bacteria was studied by many scientists, and it was proved that the silver nanoparticles are the powerful weapons against the MDR bacteria such as *Pseudomonas aeruginosa*, ampicillin-resistant *Escherichia coli*, erythromycin-resistant *Streptococcus pyogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA).

Silver nanoparticles have important biological properties as follows: they are effective bactericidal agents against broad spectrum of bacteria, including antibiotic resistant strains, fast-acting fungicide against common fungi including *Aspergillus*, *Candida* and *Saccharomyces*. Silver nanoparticles of 5–20 nm diameters can inhibit HIV-1 virus replication. [4].These can not only alter the expression proteinases, which are important in inflammatory and repair processes, but also suppress tumour necrosis factor (TNF), interleukin (IL)-12 and IL- 1b and induce apoptosis of inflammatory cells. Moreover, silver nanoparticles are also responsible for cytokine modulation in wound healing and inhibition of the biofilm formation.

Silver nanoparticles are used as effective antimicrobial agents. They have bactericidal potential against MDR

organisms. Colloidal silver nanoparticles are found to possess significant bactericidal potential against MRSA and Gram-positive and Gram-negative bacteria. Gram-negative bacteria include members of the genera *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella* and *Vibrio*. Gram-positive bacteria include *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus* and *Streptococcus*. Antibiotic-resistant bacteria include methicillin-and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA) and *Enterococcus faecium*, by preventing biofilm formation, which act as efficient barriers against antimicrobial agents and the host immune system to protect the bacterial colony.

Nanoparticles (NPs) are becoming widespread for their use in consumer products and medical applications; with potential for utilization as therapeutic compounds, transfection vectors, anti-microbial agents and fluorescent labels. Silver NPs are the most commercialized and prominent group of nano-compounds, attributed to their diverse applications in the health sector due to their physical as well as biological properties. Silver, in a colloidal form, is used for the treatment of bacterial infections in open wounds, and preparation of ointments, bandages and wound dressings [5]. Additionally, nanosilver has been used as a contraceptive, and marketed as a water disinfectant. Silver NPs are now being exploited for the treatment of various diseases such as retinal and acquired immunodeficiency syndrome as a result of human immunodeficiency virus [6]. Additionally, AgNPs are well known for their anti-microbial properties and are used as antiviral agents against hepatitis B, herpes Simplex virus type 1, monkey pox virus and respiratory syncytial virus [7]. Concerns on environmental exposure to AgNPs have initiated toxicity studies. Silver NP-hydrogel induced DNA damage and the production of reactive oxygen species (ROS) in cultured HeLa cells [18]. A study using human lymphocytes revealed that AgNPs caused DNA damage and cell death. Additionally, AgNPs induced oxidative stress and caused impairment of nuclear DNA in Swiss albino mice. Recently, the use of AgNPs as anti-cancer agents has proved promising. Various attempts to incorporate AgNPs into cancer treatments have been made, with positive outcomes. Although the induction of oxidative stress

by AgNP induced mt damage has been observed as the general mode of AgNP toxicity, mechanistic pathways remain unclear [8].

**2.2 Platinum nanoparticles**

The medicinal use of platinum is mainly focused on platinum compounds, but not nanoparticles. The best known platinum compound for anticancer agents is cisplatin (cis-diaminedichloroplatinum) [9]. Platinum nanoparticles (PtNPs) have recently been used for cancer treatments as well. Porcel *et al* showed that PtNPs strongly enhanced the biological efficacy of radiation. The combination of fast ion radiation (hadron therapy) with PtNPs resulted in enhanced DNA strand breakage. Fast carbon ion irradiation of platinum led to the production of radicals that amplified the lethal damage to DNA. In another study, human colon carcinoma cells (HT29) showed a concentration- and time-dependent response when exposed to PtNPs [10]. The efficacy comes from the PtNPs adsorbing the intracellular glutathione (GSH), causing levels to be reduced, while the soluble Pt species impair DNA integrity.

**2.3. Zinc oxide nanoparticles**

Zinc oxide (ZnO) has some similar properties to $TiO_2$ (i.e. its nanoparticles scatter light so it can be used for transparent UV filters, in creams or coatings). Like $TiO_2$, it is used for solar photocatalytic remediation but, compared to $TiO_2$, it has a weaker photocatalytic effect. Zinc oxide also suffers from the same limitation of absorbing only a fraction of the solar spectrum so research is underway to increase its photoresponse.

A peculiarity of ZnO is that it has a tendency to grow in self-organised nanostructures. By controlling crystal growth conditions, a variety of crystal shapes are possible. Researchers have been able to grow nanoscale wires, rods, rings, etc. The bactericidal mechanism of ZnO NPs is complex and still under investigation. However, it is believed to involve the release of $Zn^{2+}$ ions leading to the generation of hydrogen peroxide [11]. Huang *et al* showed that ZnO NPs attached to the cell walls of both gram-positive and gram negative bacteria, resulting in membrane disorganization, elevated membrane permeability, and cell damage [12]. Biocidal effects and cellular internalization of ZnO NPs on *E. coli* were also reported. ZnO NPs were synthesized in di (ethylene glycol) (DEG) medium through the hydrolysis of ionic

Zn salts. *E. coli* cells were damaged, showing membrane disorganization of gram-negative triple layer units after the contact with DEG and ZnO. This behavior caused an increase of membrane permeability, leading to accumulation of ZnO NPs in the bacterial membrane as well as cellular internalization of NPs. In another study, bactericidal effects of ZnO NPs (bare and thioglycerol (TG)-capped ZnO NPs) were also confirmed to result from membrane lipid peroxidation caused by ROS which is generated during the interaction of ZnO NPs with the culture medium [13]. Reactivity of nanoparticles toward bacteria depends on their size and shape. In general, toxicity is inversely proportional to particle size. Just as smaller-sized AgNPs release more $Ag^+$ ions against *E. coli*. Recent results suggest that the surface charge of nanoparticles affects the toxicity of AgNPs. Negatively charged nanoparticles do not adsorb to negatively charged cell membranes due to electrostatic repulsion, thus their cellular internalization is greatly inhibited [14].

## 3. Applications of inorganic Nanoparticles as therapeutic agents

Recent advances in nanotechnology are expected to help solve many key issues in biological disorders. In fact, many functional elements of biological systems are at the nanometer scale; therefore, nanomaterials can be ideally sized to assume some biological functionality at the molecular level [15]. Furthermore, nanomaterials with a size of 2–100 nm exhibit unique electronic, optical, chemical, and magnetic properties distinct from larger particles of the same material [16]. Therefore, biological phenomena can be explored by precisely controlling and harnessing these unique properties of nanomaterials, and various functional nanomaterials have been extensively applied to biomedical areas, including imaging, diagnosis, and therapy. Nanoparticles have been investigated as potential drug and gene delivery systems because they can overcome some intrinsic problems of drug efficacy by allowing targeted delivery and passage through biological barriers [17]. Active targeting can be achieved through conjugation with molecules such as folic acid (FA) or methotrexate (MTX) for recognition by the folate receptor, which is overexpressed on the surface of many cancer cells, or peptides such as arginine–glycine–aspartate (RGD) for targeting

integrins on the tumor endothelium [18]. Furthermore, various tailored nanomaterials, including core–shell structured nanoparticles or mesoporous structured nanoparticles, can perform additional functions related to imaging or controlled drug release. Considering the size-dependent physicochemical properties of nanomaterials along with their demonstrated ability to interact with biological systems, inorganic nanoparticles are promising candidates for biomedical applications [19]. Inorganic nanoparticles are formed by the crystallization of inorganic salts, forming a three-dimensional arrangement with linked atoms. The nature of the binding atoms is mainly covalent or metallic. These particles are highly ordered and rigid with little influence by the body. Organic nanoparticles, on the other hand, are mainly formed by spontaneous aggregation, as with micelles or vesicles. These systems are dynamic due to the weak nature of the cohesive interactions. Therefore, the size and geometry of organic aggregates are difficult to maintain below a certain size threshold, particularly in living systems. Although colloidal metals have been used in medicine since ancient times, their action mechanisms have been elucidated very recently. Now colloidal metal nanoparticle-based therapeutics is again attracting attention as an alternative to organic therapies in clinical settings. The development of highly uniform and biocompatible inorganic nanoparticles with optimized functional properties is critical. In the past decades, various inorganic nanoparticles have been successfully prepared by many different synthetic methods. One is the precipitation of salts in aqueous media [20]. Through this method, it is possible to synthesize a large number of nanoparticles of metals and oxides in a very simple and inexpensive manner, although it is hard to achieve good particle crystallinity and consistent size control. A second method, the hydrothermal process, uses water as a solvent and utilizes high pressure and temperature to increase the solubility of the precursors and reduce the reaction time. This synthesis can be performed above or below the supercritical point of water.

**Conclusion**

Antimicrobial agents must be effective, safe and can be used for the cure of multidrug-resistant microbial infections.

A number of antimicrobial nanoparticles have proven their effectiveness for treating antibiotics resistant infectious diseases. The behavior and fate of nanoparticles in vivo is hard to predict due to the complexity of biological systems. Systematic evaluation of whole-body effects by considering exposure concentration, accumulation and excretion, tissue and organ distribution, and potential chronic effects needs to be undertaken.

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**Effect of different media on the growth, yield and quality of water spinach under container gardening**

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**Abstract**

The increasing use of chemical fertilizers to grow vegetables such as spinach has caused numerous problems related to the environment and human health. Hence, using organic fertilizers including compost and vermicompost can be a more suitable alternative. Making use of agricultural waste and returning them to the cycle of nature rather than burying or burning them is an effective approach to help the environment. Soil organic matter. affects the chemical and physical properties of the soil and its overall health. Its composition and breakdown rate affect: the soil structure and porosity; the water infiltration rate and moisture holding capacity of soils; the diversity and biological activity of soil organisms; and plant nutrient availability. Few foliage or flowering plants are grown in field soil. Most are produced in a soilless growing medium which consists of components such as peat moss, wood residues, sand, etc. These media are designed to provide the necessary aeration, drainage and water holding properties required for plant growth in a container. An ideal potting medium should be free of weeds and diseases, heavy enough to avoid frequent tipping over and yet light enough to facilitate handling and shipping. The media should also be well drained and yet retain sufficient water to reduce the frequency of watering.

**Introduction**

Water spinach (*Ipomoea aquatica* Forsk) locally known as *Karmatha bhaji* belongs to family convolvulaceae. It has a short growth period and can be cultivated either in marshy land or flooded soils. Moreover, it has been found that water spinach has a high potential to convert nitrogen from biodigester effluent into edible biomass with high protein content (Sophea and Preston, 2001).

Water spinach is an herbaceous aquatic or semi-aquatic perennial plant of the tropics or subtropics. It's leaves are flat, and vary in shape depending on genotype, from heart-shaped to long, narrow and arrow-shaped. Narrow leaves are 1-2.5 cm wide and 20-30 cm long. The large,

attractive flowers have the typical open, trumpet shape of convolvulus or bindweed flowers. There are two major cultivars of water spinach,

- Ching Quat (known as "green stem") – this has a narrow, pointed leaves and white flowers and is adapted for moist soils. This can be grown in beds, provided there is always plenty of moisture.
- Pak Quat (known as "white stem") – this has broad, arrow-shaped leaves and pink flowers. It is adapted to aquatic conditions and also called "Water Ipomea".

A container in gardening is a small, enclosed and usually portable object used for displaying live flowers or plants. It may take the form of a pot, box, tub, pot, basket, tin, barrel or hanging basket.

**Effect of different media on growth parameters of water spinach**

Lal *et al.* (2002) studied the effects of farmyard manure (FYM; 0, 50 and 100 t/ha$^{-1}$) and irrigation (4, 5, 6 and 7 cm) on the growth and yield of onion cv. Hisar-2. Plant height, number of leaves plant$^{-1}$, bulb size and bulb yield increased with increasing rates of farmyard manure and irrigation. The interaction effects between FYM and irrigation were significant only for bulb size and yield.

Magnani *et al.* (2003) evaluated the growth rate and qualitative characteristics of 3 vegetable (lettuce, cabbage and cauliflower) seedlings, grown with an organic method, were evaluated. The organic method consisted of using coco-peat as the growth medium and organic fertilizer for fertigation. This method was compared with a traditional one based on a peat growth medium and synthetic fertilizers for fertigation. The results showed different responses among the vegetables, regarding growth rate and quality. Lettuce grown with organic method presented an increase of growth rate, fresh weight, leaf number and area, height, root/shoot ratio and nutrient content compared to the traditional method. On the contrary, cabbage and cauliflower, grown with organic method, showed a reduction of growth rate, dry weight, leaf number and area, chlorophyll content, height and nutrient content.

Reddy and Mallareddy (2004) studied the acclimatization of 4 parwal (*Trichosanthes dioica*) genotypes, *i.e.* Swarna Alaukik, elite selection-1, Swarna Rekha and male, studied in different substrates, i.e. vermiculite, soilrite, coco-peat, coco-peat+sand in 3:1 and 2:2 ratio, and sand+vermiculite+coco-peat in 1:1:1 ratio, in the greenhouse. Swarna Alaukik

recorded the highest survival percentage (89.94%), followed by elite Selection-1 (85.71%) and Swarna Rekha (81.42%), in coco-peat, whereas Male survived better in vermiculite (64.28%). Shoot length (10.06 cm) and number of leaves (7.97) were higher in coco-peat than in any other substrate after 28 of growing period.

Anshebo *et al.* (2004) observed high heritability estimates for vine traits viz., length of vine, number of branches plant$^{-1}$ and weight of foliage plant$^{-1}$. The least estimate of heritability was observed for number of tubers plant$^{-1}$. The characters such as number of branches plant$^{-1}$, weight of single tuber, girth of tuber and length of tuber showed high heritability estimates associated with high genetic advance indicating the presence of additive gene effect.

Arcidiacono *et al. (*2005) conduct trial on different substrates for soilless cultivation in an open system were compared. The experimental protocol includes three randomized blocks made of six rows of pots filled with six different substrates: Etna lapillus, expanded clay, perlite, peat, cocopeat, and sand. Then, tomato plants were planted in each pot. In the central pot of each row, thermoresistance probes were installed at different locations in the substrates in order to monitor the temperature of the media. The total solar radiation, the air temperature and relative humidity were measured inside and outside the greenhouse. The net radiation was also measured inside the greenhouse just above the substrates. The effect of the nature of the different substrates on their thermal regimes was examined on the basis of the collected data. Moreover, the connections between the substrate temperatures and the microclimatic variables were analysed.

Botrini *et al.* (2006) studied the growth rate and quality of tomato seedlings grown on 2 cocopeat growth media, with organic fertirrigation, and the effects of these techniques on seedling development after transplanting were evaluated. The daily increase in fresh weight and height of seedlings grown on cocopeat supplemented with borlanda and natural fertilizers were similar to those of seedlings grown with a traditional system based on peat and synthetic fertilizers. At the time of transplanting, the qualitative parameters of the seedlings were the same for the traditional growth system and the organic system based on cocopeat with borlanda and natural fertilizers.

Engida *et al.* (2007) studied high heritability and expected genetic advances

were recorded for vine length, vine inter node length, leaf area, above ground fresh and dry weights, number of storage root plant$^{-1}$, individual storage root weight, storage root fresh yield plant$^{-1}$.

Singh *et al.* (2008) reported significant increase in vine length, number of branches per plant and fruit yield with the application of FYM and gypsum during the two years of experimentation. Maximum fruit yield (195.2 qha$^{-1}$) was observed at 20 t FYM and with the application of gypsum @ 100% GR (gypsum requirement). The yield under canal irrigated check was found to be 214.6 q ha$^{-1}$.

Bhat *et al.* ( 2013) studied on a suitable growing substrate for organic greenhouse vegetable production. A number of combinations of vermicompost, coco-peat, sphagnum peatmoss, perlite, farm yard manure and avicumus were compared with ready-to-use organic substrate for producing tomato, cucumber and capsicum under greenhouse conditions. Vegetative growth parameters (average plant height, number of leaves, chlorophyll index) and fruit yield plant$^{-1}$ were used to evaluate various growing substrates. overall, substrates containing vermicompost, coco-peat, perlite and sphagnum peat moss (2:1:1:1 or 1:1:1:1 v/v) produced significantly better growth, yield and quality in tomato, cucumber and capsicum than other substrate combinations and in some cases were better than ready to use mixes and conventional soil based growing system.

Khan *et al.* (2013) found that growth parameters including number of flowers plant$^{-1}$, number of fruits plant$^{-1}$ and fruit diameter were also found significantly different by the application of FYM and K fertilizer. The mineral nutrition of tomato showed significant effect of FYM and K levels on plant P and K. FYM and K levels also significantly improved soil K content. It may be concluded that the Potassium applied @ 120 kg/ha along with the FYM was effective in improving the tomatoes attributes as well soil K content.

Luo *et al.* (2015) reported that the germination rates of water spinach decreased with increasing biochar rates when biochar was added alone (76.9%–83.7%), whereas the rates increased to 83.6%–85.8% when biochar was added in combination with sap. Growth parameters of water spinach and nutrient uptake by shoots and roots increased with increasing biochar rates and reached the maximum values at the biochar rate of 100 ml litter. There were significant cubic relationships between the

uptake of nutrients (N, P, and K) and biochar rates, both with and without SAP addition. In order to avoid negative effects on plant growth, the biochar application rate should be controlled at an optimal level (100 ml litter). The SAP addition not only enhanced the positive effects of biochar application on the properties of the substrate, but also inhibited the excessive rise of pH and EC following biochar additions, which led to better plant growth and enhanced nutrient uptakes by water spinach.

**Effect on different media on yield water spinach**

Sophea and Preston (2001) conducted experiment to evaluate the effect of different fertilizing practices on water spinach (*Ipomoea aquatica, var. reptans*) yield. The crop was located on a sandy, poor soil derived from alluvial deposits (pH 5.45, N 0.13%). There was no difference in fresh biomass yield of water spinach between the two treatments with bio digester effluent (17.6 and 18.6 t $ha^{-1}$, for total N and ammonia N, respectively).

Hoang *et al.* (2005) studied to evaluate the response of water spinach to fertilization with increasing levels of nitrogen (0, 10, 20, 30, 40, 50, 60 kg N/ha over 28 days) in the form of earthworm compost or urea. The biomass yield response to fertilizer N was positive and curvilinear and was greater for the earthworm compost at the higher levels of application of N. Increasing application of fertilizer N provoked linear responses in DM content, which decreased, and in crude protein content, which increased. Soil fertility was improved by the worm compost, but not by urea, as measured by the organic matter, phosphorus and potassium contents of the soil at the end of the trial. It appears that the most economical level of N is 40 kg/ha applied over the 28 day growth period.

Dixit and Kumar (2006) studied the effect of farmyard manure (FYM) and macronutrients on the yield and nutrients uptake by garlic. The treatments include: absolute control, control (FYM alone), 100% N, 100% NP, 100% NPK, 100% NPKS, 100% NPKSMg, 125% N, 125% NP, 125% NPK, 125% NPKS, 125% NPKSMg, 150% N, 150% NP, 150% NPK, 150% NPKS and 150% NPKSMg. Results showed that in general, the 150% N.P.K.S.Mg recorded the maximum garlic bulb yield and increased nutrients (N, P, K, S and Mg) uptake.

Bohme *et al*. (2014) reported that the spinach yield was in average approximately 20% higher on rockwool than on coco-peat mainly due to a higher number of fruits harvested but the fruits on rockwool were also longer than on coco-peat. Regarding the quality in the fruits grown on coco-peat a higher mineral content, in particular K, P and Mg, was determined. In both substrates significant differences between the genotypes of bitter gourd.

Nunal *et al.* 2014. conducted the effectiveness of cocopeat and rice hull powder obtained from agricultural wastes as biocarriers for an oil-degrading bacterial consortium. Scanning electron microscopy revealed colonization and strong attachment of bacterial cells on the surface of both carriers. Results of a 60-day in vitro seawater bioremediation trial showed significant oil reduction and high cultivable bacterial counts in treatments augmented with the carrier-attached bacterial consortia compared to treatments supplemented with the same consortium in free living and encapsulated forms. Significant degradations in both aliphatic and aromatic fractions were obtained in treatments augmented with carrier-immobilized consortia. The developed immobilized cells showed sustained activities and viabilities during storage for six months. Results of this study demonstrated that inexpensive waste materials can be utilized as biocarriers of an oil-degrading consortium and that immobilization on biocarriers can enhance the bioremediation of oil-contaminated seawater.

Surrage *et al.* (2010) revealed that Forterra Royal GRO 1 (GRO 1; coconut coir/vermicompost) and Forterra Royal GRO 2 (GRO 2 aged pine bark/ coconut coir/vermicompost) attained significantly higher marketable yields plant$^{-1}$ compared with the plants grown in RW. A similar trend was seen in the incidence of Blossom End Rot (BER) with GRO 1 and GRO 2 having reduced numbers of BER incidences per plant when compared with RW. In conclusion, the addition of vermicompost to organic growing substrates is beneficial for tomato growth and yield.

Hegde and Reddy (2012) study the rapid regeneration protocol of brinjal was conducted. The shoot tip and hypocotyl explants from the in vitro grown sterile seedling were used for regeneration. In hardening, highest survival percentage (100%) and healthy growth of plantlets were observed in mixture of vermiculite, farmyard manure and coco-peat in 1:1:1 ratio.

Khan *et al.* (2013) In vestigate the results indicated that application of FYM and various K levels had significant effect on the growth, yield and nutrient content of tomatoes. The highest yield of tomatoes was (39.05 t/ha) observed in the pots receiving FYM and 41.97 t $ha^{-1}$ was found in the treatment receiving K @ 120 kg/$ha^{-1}$.

Sharma *et al.* (2014) studied the treatments consisting of two levels of vermicompost (0, 2.5 t $ha^{-1}$), three levels of potassium (0, 10 kg $ha^{-1}$, 20 kg $ha^{-1}$) and three levels of iron (0, 20 kg $ha^{-1}$, 40 kg $ha^{-1}$) were applied to crop *Trigonella foenum-graecum* var Rmt-1 as soil application. Results showed that application of vermicompost, potassium and iron individually and in combination significantly influenced the yield attributes and yield of the crop during both the years.

**Effect on different media on Economic of water spinach**

Umar *et al*. (2007) carried out analysis of the nutritional composition of water spinach (*Ipomoea aquatica* Forsk) leaves were carried out using standard methods of food analysis. The proximate composition as well as mineral elements was determined. The leaves were found on dry weight basis to have high moisture (72.83+or-0.29%), ash (10.83+or-0.80%), crude lipid (11.00+or-0.50%), crude fibre (17.67+or-0.35%) and available carbohydrate (54.20+or-0.68%), but low in crude protein content (6.30+or-0.27%). The leaves also have energy value (300.94+or-5.31 kcal/100 g) that is within the range reported in some Nigerian leafy vegetables. The mineral element contents were high with remarkable concentration of K (5,458.33+or-954.70 mg/100 g) and Fe (210.30+or-2.47 mg/100 g). Also the leaves content moderate concentrations of Na (135.00+or-2.50 mg/100 g), calcium (416.70+or-5.77 mg/100 g), Magnesium (301.64+or-12.69 mg/100 g) and P (109.29+or-0.55 mg/100 g), with low Cu (0.36+or-0.01 mg/100 g), Mn (2.14+or-0.22 mg/100 g) and Zn (2.47+or-0.27 mg/100 g) contents. Comparing the mineral content with recommended dietary allowance, it was showed that the plant leaves is good sources of K, Mn and Fe for all categories of people, while Mg is adequate enough for adult female and children. From the result, Ipomoea aquatica Forsk leaves could be used for nutritional purposes, due to the amount and diversity of nutrients it contains.

Qiuzhuo *et al.* (2014) studied on water spinach which was grown on aquatic eco-nomic crops floating bed which harvested at regular time intervals. The total

harvest weight of water spinach was 11,907 kg, which could achieve good economic benefits. A small parts of the water spinach were transplanted to crab ponds. The transplanted water spinach could not only serve as food source for crab, but also purify the water quality in crab ponds. Based on efficient utilization and recycling of natural resources and harmless discharge of wastes, the concept of circular economy was applied to agriculture system, and a recycling and utilization mode of solid waste in ecological agricultural park was realized, which could provide a good sample for sustainable development in ecological agricultural park.

Malakar *et al.* (2015) reported that human race is dependent on the use of traditional plant-based medicines as well as poly herbal preparations. And from the last few decades several research works are carried out which confirms the potentiality of these natural sources as a good source of medications. *Ipomoea aquatica* was among such plant having good nutraceutical applications and is commonly consumed as a vegetable and is commonly found in tropical Asia, India, Africa and Australia, etc. The plant is considered to be a good source of vitamins, minerals, plant proteins, fibers, etc. as well as the plant is supposed to have tremendous pharmacological importance. The present review aims to present a brief overview of the medicinal use as well pharmacological value of the plant.

Shamli and Chandra (2015) studied the natural products, such as plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. *Ipomoea aquatica* Forsk (IAF), commonly called water spinach, belongs to the family Convolvulaceae. The present study reveals the antibacterial and phytochemical analysis of various organic extracts (acetone and petroleum ether) of leaves of plant of *Ipomoea aquatica* forsk. Acetone and Petroleum ether extracts of *Ipomoea aquatica* Forsk were tested against four common bacteria of medical importance using Disc Diffusion (DD) and Well Diffusion (WD) assay. Acetone extract showed the maximum zone of inhibition when compared with petroleum ether extract in both disc diffusion and well diffusion method. Phytochemical analysis of both the extracts showed the presence of carbohydrates, phenols, tannins, proteins and terpenoids. The results obtained in the present study indicate that *Ipomoea aquatica*

could be a good source of anti-bacterial drug, relatively safe for consumption.

Southavong *et al.* (2016) investigated the effect of biochar, charcoal and biodigester effluent on growth of water spinach soil amender (biochar or charcoal or none) at 40 t $ha^{-1}$ and level of effluent (0, 25, 50, 75 or 100 kg N $ha^{-1}$) applied to samples of soil held in fifteen litre capacity plastic baskets. Sixty seeds of water spinach were planted in each basket. After germination, some seedlings were removed to balance the number in each basket (40 seedlings) for the rest of the experiment. The plants were irrigated every morning and evening. Measurements were made of height, number of leaves, and weight of aboveground biomass after 28 days and again (regrowth) after a further 28 days. Both soil menders (biochar and charcoal) gave similar improvements in water holding capacity, from 27.4% to 39.0 and 37.6, respectively. Soil pH was increased from 4.7 to 6.6 due to addition of biochar and to 6.3 with charcoal. Biochar increased foliage yield of the water spinach in both the first and second harvests, but there was no apparent effect on foliage growth from application of charcoal. In the first harvest, there were curvilinear responses to biodigester effluent for biochar and charcoal amenders, with the peak occurring at between 50 and 75 kg N $ha^{-1}$. For the unamended soil the response was linear with the highest yield at 100 kg N $ha^{-1}$. In the second harvest, the response to effluent for the biochar amender was again curvilinear with the peak at 5075 kg N $ha^{-1}$ by contrast the response to effluent with the charcoal amender was linear with maximum yield requiring 100 kg N $ha^{-1}$. On the un amended soil there was no relationship between effluent level and biomass yield.

## Conclusion:

Growing media are used in smaller quantities and have a great role in the fertilizer program to achieve higher and sustainable crop yields. There is enormous potential for container gargening systems to utilize organic waste products from other industries and, atthe same time, recycle valuable nutrients.

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# Antimicrobial and phytochemical studies on twelve medicinal plants against multi-drug resistant *Staphylococcus aureus*

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## Abstract

Phosphate Buffer Saline extracts of 12 Indian medicinal plants traditionally used in medicine were studied for their antimicrobial activity against *Staphylococcus aureus* of clinical origin. Of these, 09 plant extracts showed varied levels of antimicrobial activity against one or more test bacterial strains. Qualitative phytochemical tests of certain active extracts demonstrated the presence of common phytocompounds in the plant extracts including phenols, tannins and flavonoids as major active constituents.

*Keywords*: Medicinal plants; Phytochemicals, Antimicrobial activity; Multidrug resistance; TLC-bioautography

## 1. Introduction

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world [1-5]. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immune-compromised, AIDS and cancer patients [6-7]. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new active compounds for which bacteria may not develop resistance easily and also which should be safe to human use.

## 2. Materials & Methods

### 2.1. *Phytochemical analysis*

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by Harborne *et al*. [8a & b]. The plant extracts were screened for

the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids.

2.2. *Antimicrobial assay*

Antibiotic sensitivity of test strains was determined by the standard Disc diffusion method of Bauer et al. (1966) against a number of antibiotics. All antibiotic discs were purchased from the Hi-Media Pvt. Ltd. (Bombay, India) [9]. The agar well diffusion method [10] with slight modifications was used and accordingly 0.1 ml of diluted inoculum ($10^5$ CFU/ml) of test organism was spread on MHA plates. Wells of 6 mm diameter were punched into the agar medium and filled with 40 microlitre (150 mg/ml) of plant extract, solvent blanks and antibiotic (vancomycin, 30 mg/ml conc.) to which the test bacteria were sensitive. The plates were incubated for 18 h at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

## 3. Results and discussion

Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. The expanding global problem of antibiotic resistance can be controlled and resolved by the use of such natural agents which have great promising antimicrobial potential against MRSA and are safe to use with no side effects that are often associated with synthetic drugs. Fleming's serendipitous discovery of penicillin was the start of exploitation of the most prolific natural source. From plants, the breakthrough in the area of antimicrobials has been realized.

Indian traditional medical systems such as Homeopathy, Ayurved, Unani medicines used by native groups for curative purposes documented the therapeutic efficacy of herbal products. Further support for the suggestion that natural products contain a wealth of biologically active molecules comes from the results obtained by several researchers during antimicrobial testing of these products against different microbes. In the present study phosphate buffer saline extracts of 12 traditionally used Indian medicinal plants have been tested against drug-resistant bacteria. Plant parts used along with their code number are given in Table 1.

**Table 1: Name of Plant and its parts used along with their code number**

| Denotion | Name of Plant | Plant |
|---|---|---|

| | | **parts used** |
|---|---|---|
| **1** | *Calendula officinalis* | Flower |
| **2** | *Rosa indica* (*Rosa indica*) | Leaves |
| **3** | *Chrysanthemum coroneriyum* | Leaves |
| **4** | *Calendula officinalis* | Leaves |
| **5** | *Polygonum bistorta* | Leaves |
| **6** | *Prunus dulcis* | Leaves |
| **7** | *Swetia charita* | Leaves |
| **8** | *Helianthus annuus* | Leaves |
| **9** | *Azadirachta indica* | Leaves |
| **10** | *Jasminum sambac* | Leaves |
| **11** | *Psidium guajava* | Leaves |
| **12** | *Emblica officinalis* | Leaves |

**Table 2: Antimicrobial activity of recovered isolates against different plant extracts (PBS Extracts)**

| | **Mean Zone Diameter (In mm)** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Samples** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **N1** | **R** | **R** | **16mm** | **R** | **11mm** | **22mm** | **R** | **R** | **15mm** | **14mm** | **R** | **11mm** |
| **N3** | **R** | **16mm** | **R** | **R** | **R** | **R** | **R** | **R** | **R** | **R** | **R** | **10mm** |
| **N6** | **R** | **R** | **R** | **R** | **R** | **14mm** | **R** | **13mm** | **R** | **R** | **12mm** | **15mm** |
| **S3** | **R** | **R** | **15mm** | **14mm** | **R** | **15mm** | **R** | **R** | **20mm** | **R** | **12mm** | **18mm** |
| **E4** | **R** | **R** | **R** | **R** | **12mm** | **16mm** | **R** | **R** | **17mm** | **R** | **R** | **R** |
| | | | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1 | R | R | 15mm | R | R | 19mm | R | R | 13mm | R | R | R |

**R: Resistant i.e. <10mm zone of inhibition**

The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter, respectively as given in Fig. 1. Among the 12 plant extracts, 8 plants were found to have antimicrobial activity as shown in Table 2. *Prunus dulcis* leaves extract showed activity against 5 samples, *Azadirachta indica* and *Emblica officinalis* leaves extract showed activity against 4 samples, *Swetia charita* extract showed activity against 4 samples, *Chrysanthemum coroneriyum* extract showed activity against 3 samples, *Polygonum bistorta* and *Psidium guajava* leaves extract showed activity against 2 samples, *Rosa indica* extract, *Calendula officinalis* leaves extract and *Helianthus annuus* extract showed activity against 1-1 sample each. During Biochemical characterization, steroids, rasins and glycosides were the most common phytochemicals found in all these plant extracts. So the antimicrobial activity must be due to them.

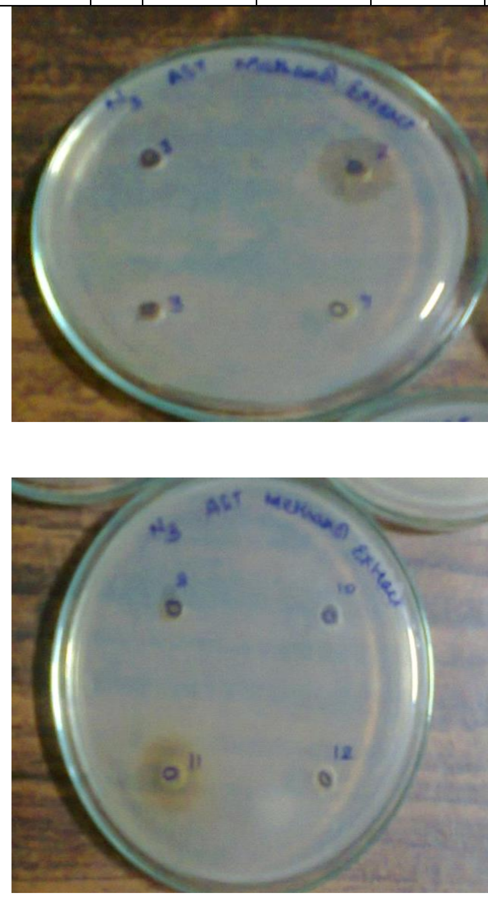

**Figure 1 showing results of antimicrobial activity of plant extracts in the form of zone of inhibition of test bacterial isolates growth**.

The bioactive compounds are usually distributed as secondary metabolites in every plant cell though leaf is found to be the highest accumulatory plant part and has high Active Pharmaceutical Ingredient (API) [11]. Antimicrobial compounds in plants are reported to be aromatic or saturated organic molecules which are easily solubilized in organic solvents. Therefore, after

preliminary screening for a better solvent, phosphate buffer saline was used for the preparation of twelve plant leaf extracts, which were selected for their antimicrobial spectrum against different microbes as claimed in various ethno-medical studies [12].

The results of screening are encouraging as out of the 12 plants, 09 extracts showed antibacterial activity against one or more test bacteria. Similar reports on antibacterial activities of certain Indian medicinal plants such as *P. granatum*, *Azadirachta indica*, and *Psidium guajava* were also reported by other workers [13-15].

Few plants in this study were also screened previously against other test strains [1, 16] and showed similar results to this study with varying degrees of potency. The difference in potency may be due to the stage of collection of the plant sample, different sensitivity of the test strains and method of extraction [15].

Phytochemical analysis of 12 active extracts demonstrated the presence of common phytoconstituents like phenols, condensed tannins, glycosides, saponins, flavonoids and alkaloids. These results are in resonance with several workers [17]. The results present here are promising; however, the knowledge of active principles involved in activity and their appropriate medicinal application is still a bit mysterious.

Present study highlights the prevalence of MRSA among various Cancer patients and the antibacterial activity of different medicinal plant extracts against it. During the study we isolated one VRSA sample also which was isolated from Pus sample of a patient who was suffering from Cancer Tumour Burger. The prevalence of VRSA is not as common till now as found in previous studies but screening of one VRSA sample in present study shows the effect of high dose of antibiotics that are given to the cancer patients which makes patients resistant to most of the antibiotics.

The present results offer a scientific basis for the traditional use of PBS extracts of *Azadirachta indica*, *Swetia charita*, *Jasminum sambac*, *Polygonum bistorta*, *Psidium guajava*, *Rosa indica*, *Calendula officinalis*, *Prunus dulcis*, *Thryhanthemum coroneriyum*, *Helianthus annuus*, *Emblica officials.* However, further studies on these medicinal plants are necessary to determine their active constituent-activity relationship. The antibacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration.

It is therefore concluded that, the plant extract possess antibacterial activity against the test organism i.e. *Staphylococcus aureus.* The zone of inhibition varied suggesting the varying degree of efficacy and different phytoconstituents of herb on the target organism. The antibacterial activity of plants may be due to the presence of various active principles in their leaves. Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing antimicrobial property. The ethno-botanical study of plant is important for modern day medicine but its usefulness cannot be overemphasized if methods are not standardized to obtain comparable and reproducible results. At present, scientists are investigating for plant products of antimicrobial properties. It would be advantageous to standardize methods of extraction and in vitro antimicrobial efficacy testing so that the search for new biologically active plant products could be more systematic and interpretation of results would be facilitated. Thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro should be subjected *in vivo* testing to evaluate the efficacy in controlling the incidence of disease in crops, plants, and humans. Efficient collaborations with pharmacologists and medical doctors, plant pathologists and micro-biologists are crucial to see the complete development of an interesting lead compound into an exploitable product. However, *in vivo* studies on these medicinal plants are warranting determining toxicity of active constituents, their side effects and pharmacokinetic properties.

The results of present investigation clearly indicate that the antibacterial activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs. Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drugs. Study of the synergistic interaction of active phytocompounds with antibiotics is required to exploit these potential plant extracts in the combination therapy of infectious diseases caused by multi drug-resistant organisms.

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